Challenges and Progress Toward Determining Pneumonia Etiology

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Challenges and progress towards determining pneumonia etiology

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The World Health Organization estimates that lower respiratory tract (LRT) infection is the most common infectious cause of death worldwide and the fourth most common cause overall, with 3 million deaths attributed to LRT infection in 2016 [1]. Timely and reliable identification of the underlying pathogen is critical for initiating effective and tailored antimicrobial treatment, but identifying the microbial etiology of pneumonia is challenging in many clinical settings. Community-acquired pneumonia (CAP) is an acute infection of the lung parenchyma acquired outside hospital or healthcare facilities. Microbiological testing to attempt an etiological diagnosis is generally recommended for CAP patients requiring hospitalization [2, 3].

The “gold standard” for determining pneumonia etiology is the detection of respiratory pathogens in specimens taken directly from the site of infection, the lungs, by bronchoalveolar lavage (BAL), pleural fluid sampling, or lung biopsy [4]. Sputum and tracheal aspirates are LRT specimens with a higher probability of upper respiratory tract (URT) contamination. Because BAL, pleural fluid sampling, and lung biopsy are invasive procedures, they are rarely performed in clinical practice. Therefore, the etiological diagnosis of CAP mostly depends on the detection of respiratory pathogens from specimens distant to the site of infection, such as URT samples, blood, and urine. Current recommendations for routine diagnostic testing in adult CAP patients include sputum Gram stain and culture, blood cultures, and urinary antigen tests for *Legionella pneumophila* and *Streptococcus pneumoniae* (pneumococcus) [2]. In children, sputum collection is hampered by difficulties with expectoration, and urinary pneumococcal antigen tests are not recommended. Testing for influenza virus, other respiratory viruses, and *Mycoplasma pneumoniae* in URT specimens by polymerase chain reaction (PCR) and/or rapid antigen tests may be done to evaluate children with CAP [3].

Recent large-scale studies performed extensive microbiological testing to determine the etiology of pneumonia in hospitalized adults (Musher et al. [5], U.S., *n*=215; CDC EPIC study [6], U.S., *n*=2,259; Gadsby et al. [7], U.K., *n*=323; CAPiTA study [8], the Netherlands, *n*=1,653) and children (CDC EPIC study [9], U.S., <18 years old, *n*=2,222; Drakenstein Child Health study [10], South Africa, <2 years old, *n*=284; PERCH study [11], Africa and Asia, <5 years old, *n*=1,769) with a positive chest radiograph. A viral or bacterial pathogen was detected in 81–99% of pediatric and 38–45% of adult CAP cases, except Gadsby et al. [7] reported a high detection rate in adults of 87%. Viruses accounted for the majority of detected pathogens [6, 7, 9-11], particularly in young children (>90%) [10]. Both viral and bacterial pathogens were detected in up to 90% of children [9-11], but <10% of adult patients [5, 6, 8]. Conjugate vaccines have successfully reduced the burden of the former main causes of pneumonia, *S. pneumoniae* and *Haemophilus influenzae* type b, over the past three decades. *M. pneumoniae* is now the most commonly detected bacterial pathogen in children hospitalized with CAP in the U.S. [9].

Test results and epidemiological data must be carefully interpreted as no single diagnostic method applied to non-pulmonary specimens has both high sensitivity and high specificity for determining pneumonia etiology. Blood cultures are insensitive because they are positive in less than one-third of suspected bacterial pneumonia cases [5, 6, 8-11], but they are highly specific in determining pneumococcal pneumonia. In contrast, whole blood PCR and urinary antigen tests exhibit poor specificity, as they are also positive in patients who carry *S. pneumoniae* in the URT [11]. *S. pneumoniae* can be detected in the URT of up to 77% and 34% of healthy children and adults, respectively [11, 12]. In addition, *S. pneumoniae* carriage elicits

systemic antibody responses to pneumococcal antigens, limiting antibody detection as a diagnostic test to reliably determine pneumonia etiology [13]. This may also be true for *M. pneumoniae*, the detection of which by PCR in URT specimens and serology is not able to differentiate between infection and carriage [14]. Furthermore, the detection of many potential pathogens in the URT of a CAP patient represents carriage, URT infection, asymptomatic infection, or persistence of the pathogen after infection [11]. This complicates the assignment of causative pathogens in the URT for the pneumonia episode. In the PERCH study [11], more than half of childhood pneumonia cases (59%) and controls (54%) had ≥4 pathogens detected by multiplex PCR in URT specimens. Only RSV and *Bordetella pertussis* were rarely detected in URT specimens from healthy controls [10, 11].

Sputum has advantages in determining pneumonia etiology, including its origin from the LRT and non-invasive collection. Sputum induction, such as through hypertonic saline nebulization, may increase the likelihood of obtaining a valid sample and is especially useful in young children who are unable to expectorate spontaneously. Interestingly, testing of induced sputum is more sensitive than testing URT samples for the detection of several CAP pathogens in young children [10].

In this issue of *Clinical Infectious Diseases*, Ogawa et al. present a rigorous systematic review and meta-analysis of the literature on the diagnostic accuracy and yield of sputum Gram stain for diagnosing a bacterial pathogen in CAP [15]. Twenty-four studies were included (*n*=4,533 adults), 22 on diagnostic accuracy and 4 on the diagnostic yield of this method. Consistent with previous studies, though on a larger scale, these new results suggest that Gram stain performed on good-quality sputum is highly specific for the diagnosis of *S. pneumoniae* and *H. influenzae* infections in

adult CAP patients (point estimates 0.91 and 0.97, respectively). Good-quality sputum was defined as the presence of <10 squamous epithelial cells and >25 polymorphonuclear cells per low-power field [16, 17]. Data on other bacteria were limited. Sputum Gram stain diagnosed the bacterial pathogens in 36% of patients when sputum samples were collected successfully, increasing to 73% if only good-quality sputum samples were selected.

This study has three major strengths. First, the analyses were performed on studies with sputum Gram stain of good-quality samples. The applied sputum quality criteria may have helped detect URT contamination [16]. Second, the different (composite) reference standard tests of studies included in the work were extracted thoroughly and considered. This is essential, as BAL, pleural fluid sampling, and lung biopsy were rarely available, and the reference standard test (usually sputum culture) was imperfect in most cases. Third, the meta-analysis was performed using the Bayesian latent-class model, which accounts for the multiple imperfect reference standards. The study's major limitations are variations between included studies regarding pre-test symptoms and treatment, sample collection, transport, and processing methods, as well as the interpretation of results. Therefore, failure to detect pathogens on sputum Gram stain does not necessarily prove their absence. However, summary estimates across different subgroup analyses were consistent with those of the main analysis, and the major findings were confirmed by another recent review [18].

The results of this meta-analysis support current guidelines recommending prompt examination of pre-treatment sputum Gram stain and culture in hospitalized adults with CAP if good-quality specimens can be obtained and quality performance measures met [2]. Sputum Gram stain can be clinically useful in narrowing standard

(empirical) treatment decisions by providing immediate information about the potential causative pathogens, and sputum culture may enable pathogen isolation for sensitivity testing, which is the most important issue for many patients. Future studies on sputum Gram stain may focus on the possibility and consequences of URT contamination of even good-quality sputum and further elaboration of the diagnostic utility of induced sputum in children [10, 19-21].

Progress has been made towards determining the etiology and pathophysiology of pneumonia in recent years. Future challenges facing pneumonia etiology research include assigning a causative agent(s) from multiple potential pathogens detected in the URT during a pneumonia episode (even more so in children than adults), improving the pathogen-detection yield in adults, and tackling the pathophysiological significance of the lung microbiome, including putative pneumonia pathogens, moving away from a single-pathogen perspective [22, 23]. This can only be achieved with improved diagnostic methods. Thus, we need to advance current testing methods while exploring new and innovative approaches. Such an innovative and minimally invasive approach may be the detection of pathogen-specific antibody-secreting cells **(Figure 1)** [24], which allow differentiation between *M. pneumoniae* infection and carriage in children with CAP [14]. Other promising diagnostic approaches are exhaled breath analysis [25], novel biomarkers [26], new point-of-care and antigen detection assays [27], multidimensional (molecular) assessment of the host response [28], and new analytical approaches [11]. Efforts to determine the microbial etiology and understand the complex pathophysiology of pneumonia are key to reducing antibiotic overuse and resistance.

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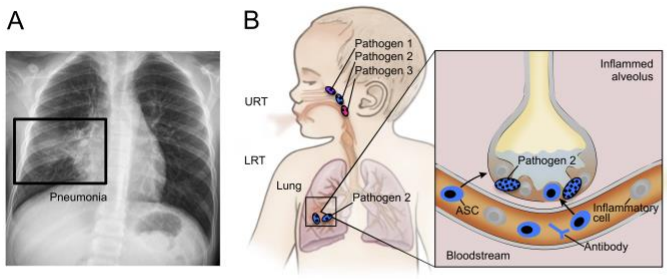
Figure 1. Pneumonia pathophysiology and testing methods. *A:* Chest radiograph of a child with CAP. The pulmonary infiltrate is indicated by the frame. *B:* Schematic representation of the possible pathophysiology of childhood pneumonia. *C:* Overview of different specimens and testing methods for pneumonia diagnostics. Abbreviations: ASC, antibody-secreting cell; BAL, bronchoalveolar lavage; ELISA, enzyme-linked immunosorbent assay; ELISpot, enzyme-linked immunospot; LRT, lower respiratory tract; PCR, polymerase chain reaction; URT, upper respiratory tract.



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Body site	Specimens	Test methods
URT	Pharyngeal swab Nasopharyngeal swab/secretion	PCR Antigen detection assay
LRT	Sputum Tracheal aspirate BAL Lung biopsy Pleural fluid (Exhaled breath)	Gram stain Bacterial culture PCR Antigen detection assay (Mass spectrometry)
Blood	Whole blood Serum	Bacterial culture PCR ELISA (ASC ELISpot)
Urine	Native urine	Antigen detection assay